IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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AFFIDAVIT
(37 CFR 1.132 and MPEP 2164.05)

1. I , Vipin Kumar, declare as follows: I received my Ph.D. at the Indian Institute of Science, Bangalore, in 1986 and had postdoctoral research fellowships at Harvard (1985-1987), the California Institute of Technology (1987-1991) and at the University of California at Los Angeles (1991-1993). I was formerly on faculty at the University of California at Los Angeles (1993-1997) and La Jolla Institute for Allergy and Immunology (1997-2002). I am currently an Associate Professor at the Torrey Pines Institute for Molecular Studies. I have published approximately 60 peer-reviewed scientific articles and have two patents pending. These publications relate to immunotherapeutics, especially T-cell mediated responses. I have developed DNA-based, peptide-based and adenovirus-based vaccines for autoimmune diseases and cancer. I am a person of ordinary skill in the art.
2. I have analyzed information in the presently claimed invention filed on December 21, 2000.

3. Claim 1. A vaccine useful in preventing and treating diseases caused by a pathogen capable of infecting, or avoiding destruction by, macrophages, said vaccine comprising at least one vector that comprises at least one nucleotide sequence encoding at least one antigen derived from said pathogen, and wherein said antigen is capable of generating an immune response in a recipient thereof. At the time of patent filing, Claim 1 was enabled by the specifications describing a "genetic vaccine utilizing recombinant vectors such as the adenovirus vector system and naked DNA" [0012] "comprising nucleotide sequences encoding P. acnes proteins or fragments thereof" [0012]. Additionally, references are made to pathogens that avoid immune responses, and it is indicated that cell-mediated responses may be necessary in addition to antibody responses [0007] and [0012]. The specifications describe methods that will induce cell-mediated immune responses [0010] [0012] [0016]. Also, the "Detailed Description of the Preferred Embodiments" sufficiently describes the molecular, biochemical, cellular and mammalian techniques and experimentation and materials used to create and administer the vaccine. At the time of patent filing, a person of ordinary skill in the art had the materials (vectors, enzymes, cells, etc) available and possessed the technical skill in molecular biology, biochemistry, cell biology and mammalian experimentation to create a useful genetic vaccine and administer the genetic vaccine to mammals. This is further supported by the fact that since the 1980's, there have been 509 gene therapy clinical trials in progress, which use recombinant vectors (www4.od.nih.gov/oba/rac/clinicaltrial.htm). Also, there were examples in the literature and of products on the market, which demonstrate the use of such recombinant vectors to generate immune responses effective in prevention and treatment of different human diseases, including Tacket C. O. et al.(1), Graham F.L. 2000 (2), and GlaxoSmithKline's Engerix-B® vaccine for Hepatitis B. Thus, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

4. Claim 2. The vaccine of claim 1, wherein said pathogen is *P. acnes, L. monocytogenes, S. typhimurium, N. gonorrhoea, M. avium, M. tuberculosis, M. leprae, B. abortus, C. albicans; L. major,* or combinations thereof. As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens. The same references apply as for Claim 1.

5. Claim 3. The vaccine of claim 2, wherein said pathogen is *P. acnes.* As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by *P.acnes.* The same references apply as for Claim 1.

6. Claim 4. The vaccine of claim 1, wherein said vector comprises naked DNA, a recombinant viral vector, or a combination of both. As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a vaccine comprised of naked DNA, a recombinant viral vector or combination of both, which is useful in preventing and treating diseases caused by the aforementioned pathogens as evidenced by the multitude of ongoing gene therapy clinical trials (www4.od.nih.gov/oba/rac/clinicaltrial.htm), Tacket C. O. et al.(1) and Graham F.L. 2000 (2). In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

7. Claim 5. The vaccine of claim 4, wherein said recombinant viral vector is selected from the group consisting of adenovirus, adeno-associated virus, herpes virus, vaccinia and RNA viruses. As I stated for Claim 4, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian

experimentation techniques enabled a person of ordinary skill in the art to create a vaccine, comprised of a variety of recombinant viral vectors, and which is useful in preventing and treating diseases caused by the aforementioned pathogens. As an example, since the 1980's, there have been 509 gene therapy clinical trials in progress, a majority of which use viral vectors (www4.od.nih.gov/oba/rac/clinicaltrial.htm). Specific examples of the use of these viruses as gene therapy vectors are cited in the following references: Graham F.L. 2000 (2), Tebbutt S. J.1999 (3), Cotter MA, Robertson ES.1999 (4) and Broder C. C. and Earl P. L. 1999 (5).

8. Claim 6. The vaccine of claim 5, wherein said recombinant viral vector is an adenovirus. As I stated for Claims 1 and 5, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a vaccine comprising a recombinant adenoviral vector, which is useful in preventing and treating diseases caused by the aforementioned pathogens. As mentioned previously, since the 1980's, there have been 509 gene therapy clinical trials in progress, a significant fraction of which use adenoviral vectors (www4.od.nih.gov/oba/rac/clinicaltrial.htm). Examples are cited in the following reference: Graham F.L. 2000 (2).

9. Claim 7. The vaccine of claim 1, wherein said vector further comprises a nucleotide sequence encoding an adjuvant. As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which includes adjuvants such as "cytokines" that are able to "activate ...T cells" and "mediate and regulate immune and inflammatory processes" [0016] and "co-stimulatory molecules, which synergize with cytokines in the activation of T cells."[0016]. Specific examples demonstrating the relevance of cytokines in generation of effective T-cell responses in mammals are cited in the following references: Gabaglia C. R. et al. 1999 (6) and

Stewart A. K. et al.1999 (7). In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

10. Claim 8. The vaccine of claim 7, wherein said adjuvant is a cytokine. As I stated for Claims 1 and 7, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which includes cytokines as adjuvants. The same references apply as for Claim 7.

11. Claim 9. The vaccine of claim 8, wherein said cytokine is IL-2, IL-12, or both. As I stated for Claims 1, 7 and 8, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which includes the cytokines IL-2, IL-12 or both. The same references apply as for Claim 7.

12. Claim 10. The vaccine of claim 1, wherein said antigen is a lipase gene or fragments thereof, a hyaluronidase gene or fragments thereof, a phosphatase gene or fragments thereof, or combinations of the foregoing. As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which utilizes *P. acnes* genes for lipase, hyaluronidase and phosphatase. At the time of patent filing, the gene for *P.acnes* lipase was already isolated (Miskin J. E. et al 1997) (8) and the Working Example 1 in the patent application was sufficient to enable the claim for *P. acnes* lipase; hyaluronidase and acid phosphatase genes from multiple bacteria were isolated and found to have significant sequence homologies at the nucleotide and amino acid levels which would allow cloning of the *P.acnes* gene by PCR, screening a genomic

DNA library or other commonly used molecular biology techniques (Hynes W. L. et al 2000) (9) and (Ostanin K. et al 1992) (10).

Also, as suggested in the patent specifications [0005] and at the time of patent filing, there was significant indication that these exocellular enzymes did not participate in the inflammatory response, which is responsible for inflammatory acne (Karvonen K. T. et al 1994) (11) and (Holland K. T. et al 1993) (12). In fact, Ingham, E. G. et al 1987 (13) determined that "there was no evidence to suggest a role for antibodies to *P. acnes* exocellular enzymes in the initiation of inflammatory acne."

Instead, these enzymes play a significant role in bacterial cell survival and infectivity, so neutralizing antibodies are likely to inhibit bacterial cell growth and prevent acne vulgaris (Gribbon E. M. et al 1993) (14), (Kearney J. N. et al 1984) (15), Higaki S. et al 1996 (16), (Hynes W. L. and Walton S. L. 2000) (17). Finally, these exocellular enzymes are secreted by the bacteria harbored inside macrophages, so they will be displayed on these antigen presenting cells, which will generate an effective cell-mediated response and destruction of the macrophages infected with *P.acnes* (Pamer E. G. 1998) (18). In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

13. Claim 11. A method of treating or preventing a disease caused by a pathogen capable of infecting, or avoiding destruction by, macrophages, said method comprising obtaining a vaccine comprising at least one vector that comprises at least one nucleotide sequence encoding at least one antigen derived from said pathogen; and administering said vaccine to a recipient in need thereof. As I stated for Claim 1, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and administration of the vaccine. The patent specifications mention some of the known methods of administration and drug delivery [0041 through [0057] and references are

cited herein. Also, Working Example 2 in the patent application provided enough detail to enable Claim 11.

Additional examples demonstrating the state of the art at the time of filing was sufficient to enable administration of vaccines are described in the following references: Tacket C. O. et al.(1), GlaxoSmithKline's Engerix-B® vaccine for Hepatitis B, and Stewart A. K. et al.1999 (7). In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

14. Claim 12. The method of claim 11, wherein said administering comprises routes of administration comprising oral, intravenous, intramuscular, transcutaneous, subcutaneous, aerosol, ocular, rectal, intraperitoneal, intrathecal, or combinations thereof. As I stated for Claims 1 and 11, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and administration of the vaccine by the routes stated in Claim 12. Specific examples of the use of these different routes of administration are described in the cited references: Gerdts V. et al 2000 (19), Babiuk L. A. and Tikoo S. K 2000 (20), Xiang Z. and Ertl H. C.1999 (21), Glenn G. M. et al 2000 (22), Lewis P.J. and Babiuk L. A. (23). However, ocular, rectal and intrathecal routes were not used at the time of the patent filing.

15. Claim 13. The method of claim 12, wherein administering comprises transcutaneous administration. As I stated for Claims 1, 11 and 12, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens using a transcutaneous route of administration. The same references apply as for Claims 1, 11 and 12.

16. Claim 14. The method of claim 13, wherein said transcutaneous administration comprises applying said at least one vector to a patch, and adhering said patch to skin of said recipient. As I stated for Claims 1, 11 and 12, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens using a transcutaneous route of administration of the vaccine via a patch. Examples of therapeutic patches are cited in the following references: Glenn G. M. et al 2000 (22), TRANSACT (http://home.intekom.com/pharm/boots-h/transact.html) and IOMAI (http://www.iomai.com) are two companies that have been selling and using adhesive transcutaneous patches since 1994 and 1997, respectively. Both of these technologies can be used to deliver said vaccine.

17. Claim 15. The method of claim 11, wherein said pathogen is *P. acnes, L. monocytogenes, S. typhimurium, N. gonorrhoea, M. avium, M. tuberculosis, M. leprae, B. abortus, C. albicans; L. major*, or combinations thereof. As I stated for Claims 1 and 11, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and administration of the vaccine. The same references apply as those that I stated for Claims 1 and 11.

18. Claim 16. The method of claim 15, wherein said pathogen is *P. acnes*. As I stated for Claim 15, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by *P. acnes* and administration of the vaccine. The same references apply as those that I stated for Claim 15.

19. Claim 17. The method of claim 11, wherein said at least one vector comprises naked DNA, a recombinant viral vector, or a combination of both. As I stated for Claims 1 and 11, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and administration of the vaccine. The same references apply as those that I stated for Claims 1 and 11.

20. Claim 18. The method of claim 17, wherein said recombinant viral vector is an adenovirus. As I stated for Claims 17, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and administration of the vaccine. The same references apply as those that I stated for Claims 17.

21. Claim 19. A kit comprising a container and one or more patches, wherein said patches have disposed thereon at least one vector comprising a nucleotide sequence encoding an antigen derived from a pathogen, said pathogen being capable of infecting, or avoiding destruction by, macrophages. The method of claim 13, wherein said transcutaneous administration comprises applying said at least one vector to a patch, and adhering said patch to skin of said recipient. As I stated for Claims 1, 11, 13 and 14, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens using a transcutaneous route of administration via a patch and patch kit. The same references apply for Claim 19 as for Claims 1, 11, 13 and 14, in addition, since 1997, a company called IOMAI (http://www.iomai.com) has been using transcutaneous patches, both of these

technologies can be used to deliver said vaccine. In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

22. Claim 20. An article of manufacture comprising a vaccine solution disposed within a tube, vial, bottle, can, or syringe, wherein said vaccine solution comprises a viral vector comprising a nucleotide sequence encoding an antigen derived from a pathogen, said pathogen being capable of infecting, or avoiding destruction by, macrophages. As I stated for Claims 1, 10 and 11, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry methods and techniques and manufacturing skills enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and manufacture of a vaccine solution. There were a number of companies and academic institutions with vaccine products that were in clinical trials or on the market, which manufactured their vaccines in solution. These included a cancer vaccine developed by McMaster University that was in clinical trials (Stewart A. K. et al.1999) (7), Cell Genesys, a company creating adenoviral-based cancer vaccines that were in clinical trials (http://www.cellgenesys.com/products.shtml) and GlaxoSmithKline's Engerix-B® vaccine for Hepatitis B, which was on the market. In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

23. Claim 21. The vaccine of claim 1, wherein said vaccine is in the form of an aqueous solution. As I stated for Claim 20 at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine in aqueous solution, which is useful in preventing and treating diseases caused by the aforementioned pathogens. It is generally known to those skilled in the art that vectors comprising DNA, viruses, proteins or combinations thereof, are very soluble in aqueous solution. Working Example 2 in the patent application is one example of a vaccine in the form of an aqueous solution. The same references apply as I described for Claim 20.

24. Claim 22. The vaccine of claim 1, wherein said vaccine further comprises a nucleotide sequence encoding a co-stimulatory molecule. As I stated for Claim 1 and 7, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which includes co-stimulatory molecules. Specific examples demonstrating the relevance of co-stimulatory molecules in generation of effective T-cell responses in mammals are cited in the following references: Putzer B. M. et al. 1997 (24) and Diehl L. et al 2000 (25).

25. Claim 23. The vaccine of claim 22, wherein said co-stimulatory molecule comprises a B7 protein, a CD40 protein or both. As I stated for Claims 1, 7 and 22, at the time of patent filing, the patent specifications and the availability of materials and conventional molecular biology, biochemistry, cell biology and mammalian experimentation techniques enabled a person of ordinary skill in the art to create a genetic vaccine that is useful in preventing and treating diseases caused by the aforementioned pathogens and which includes the co-stimulatory molecules, specifically the B7 family of proteins, CD40 family of proteins or both. The same references apply as for Claim 22.

26. Claim 24. A method of cosmetically improving the appearance of a person's skin who is suffering from acnes vulgaris, said method comprising the steps of obtaining a composition comprising a mixture of at least one vector that comprises at least one nucleotide sequence encoding at least one antigen derived from *P. acnes*, and a cosmetic agent; and administering said composition to said person. There were a number of skincare products on the market that contained mixtures of a cosmetic agent (improves skin color, adds moisture, provides sun protection or other similar applications) and affects the degree of acne by decreasing sebum levels in the hair follicle, inhibiting growth of *P.acnes* or other methods. These include products designed for mild acne patients, which contain benzoyl peroxide, topical tetracycline, salicyclic

acid, azelaic acid or the like. In conclusion, the combination of the patent specifications, my skills and the state of the prior art at the time of patent filing enables this claim.

27. The Amendment to Claims 1, 11, 20 and 24 states "wherein the vaccine comprises a genetic vaccine utilizing a recombinant vector or modified polypeptides, the vaccine further comprising antigens that do not cause a destructive form of acne." This amendment is contained within the patent specification in paragraphs [0012] on page 2, paragraphs [0038] on page 5 and paragraphs [0008] on page 1.

28. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 to Title 18 of the United States Codes, and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Vipin Kumar

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